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showing differentiation into wall cells and a central mass of cells like sporogenous tissue. $\times 485$.

Fig. 9.—The walls of the cells of the central mass are becoming mucilaginous and some of the nuclei are disintegrating. $\times 485$.

Fig. 10.—The mucilage cavity is formed, some nuclei still floating in the fluid contents, others being flattened against the wall. $\times 485$.

Fig. 11.—Section of an early stage of a mucilage duct in a leaf, showing cells resembling sporogenous tissue. $\times 485$.

ALTERNATION OF GENERATIONS AND SEXUALITY IN $DICTYOTA\ DICHOTOMA$

After the failure of many attempts made during the past three summers to raise *Dictyota dichotoma* (Huds.) Lamour. from the spores and fertilized eggs to maturity, I have succeeded in raising nearly 100 plants to a fruiting condition. Since all attempts to raise plants to maturity in the laboratory have failed so far, the cultures were started in the laboratory and then transferred to the harbor. The method employed was as follows. Several days before the time for the maturing of the crop of sexual cells, when therefore no eggs were present in the water to serve as a possible source of contamination, two good fruiting tetrasporic plants were gathered late in the afternoon and placed in two jars of sea water, one in each jar. Since the spores will not attach themselves to glass, oyster shells which had lain on land for several years were placed in these jars. On the following day many tetraspores had been liberated and had attached themselves to the shells. The parent tetrasporic plants were then removed.

On the day when eggs and spermatozoids were mature, several sexual plants were gathered late in the afternoon and placed in a jar of sea water with oyster shells in the bottom, as before. Abundant liberation of eggs and spermatozoids occurred the next morning, as usual, and the parent sexual plants were then removed.

Three cultures were thus obtained, one containing fertilized eggs, and the other two containing tetraspores, each of the latter containing the spores from a single plant. These cultures were placed in front of a north window of the laboratory.

After about two weeks, when the young plants had reached a height of I.5-2^{mm},⁵ one shell bearing many young plants was removed from each of these jars and suspended between posts about 30^{cm} below low water in a favorable situation in the harbor, and all plants of Dictyota in the immediate vicinity were removed.

⁵ This rate of growth is probably less than normal, since plants of Dictyota grow much more slowly in cultures than they do under natural conditions.

After about two months, on the day when general liberation of the sexual cells occurred, when therefore all sexual plants could be certainly distinguished, these three shells were brought to the laboratory and examined. Each shell bore many plants of Dictyota 1.2–15^{cm} high.⁶ Every plant was removed and examined under the microscope. The shell to which fertilized eggs had attached themselves bore 33 plants 2.5–15^{cm} high, all fruiting and all tetrasporic, as was shown by the presence of tetraspores or tetraspore mother cells on every plant. The two shells to which tetraspores had attached themselves bore 64 plants 2.2–13.75^{cm} high, all fruiting, and all sexual, and a few plants 1.2–2.5^{cm} high which were sterile. In handling these cultures many fragments were broken off; these were all examined with the microscope in order to be certain that no plant from any culture was overlooked. The evidence from these fragments agreed entirely with that given above for whole plants.

Since these cultures were placed in the open harbor, the possibility of contamination by spores or eggs floating in the water must be considered. In fact, a few specimens of several other species of algae and many animals (ascidians, worms, molluses, hydroids, etc.) did attach themselves to the shells. However, the fact that all the 33 plants on the shell to which fertilized eggs had attached themselves were tetrasporic, and all the 64 fruiting plants on the shells to which tetraspores had attached themselves were sexual, seems convincing evidence that no contamination by Dictyota spores or eggs occurred. Thus the belief in the alternation of tetrasporic and sexual generations in *Dictyota dichotoma*, previously based on cytological evidence alone, seems proven by the results of these cultures.

As was noted above, each of the cultures of sexual plants was produced from the tetraspores of a single plant. One of these cultures bore 17 plants (14 females and 3 males), the other bore 47 plants (26 females and 21 males). The tetraspores of a single plant are thus seen to produce plants of both sexes. From the proportion of male and female in the latter culture and from the fact that plants gathered in the harbor at random show males and females in nearly equal numbers, the possibility is suggested that half of the tetraspores produce males and the other half produce females. Material has been preserved for a cytological study of this plant, to discover whether sex determinants occur in this species. These and other results bearing on the process of reproduction in Dictyota will be given in a subsequent article.

This work has been done at the laboratory of the Bureau of Fisheries

⁶ This rate of growth is much less than that observed in Dictyota under other conditions.

at Beaufort, N. C. I am indebted to Hon. George M. Bowers, U. S. Fish Commissioner, for the privilege of working in this laboratory, and to the director, Mr. Henry D. Aller, for many courtesies extended to me during this investigation.

Summary

Plants of *Dictyota dichotoma* raised from fertilized eggs gave 33 tetrasporic plants and no sexual ones. Plants raised from tetraspores gave 64 sexual plants and no tetrasporic ones.

The tetraspores of a single plant produced both male and female plants, in one case in about equal numbers.—W. D. Hoyt, *The Johns Hopkins University*.

MICROTECHNIQUE FOR WOODY STRUCTURES

In the preparation of thin sections (5 μ or less) of hard tissues the celloidin method has been largely used with excellent results. The method as originally described by Plowman⁷ has been modified during the last few years at the laboratories of plant morphology of Harvard University, to meet the demands of work with special classes of hard tissues.

In reply to numerous inquiries as to the best method of preparing thin sections of woody tissues (trees and shrubs), and as to the advisability of using the celloidin technique, the following methods of treatment used in preparing slides for photomicrography, the study of wood structure, and instruction in wood technology are described.

- I. SELECTION OF MATERIAL.—In working with old and thoroughly dried material, the blocks for treatment should be cut from the interior of the piece and as far from the finely checked outer surface as possible. Green material and sapwood are often preferable in working with the woods of gymnosperms, especially with the pines, in order to prevent shredding of the transverse sections and tearing-out of the resin canals. The blocks should be cut in such a manner that the faces represent sections which are as nearly transverse, radial, and tangential as possible. In working with soft woods, larger blocks may be used to advantage than with hard woods, and in the case of extremely hard woods the transverse face particularly must be trimmed down to small dimensions.
- 2. Boiling.—The blocks should be given a very thorough boiling in water to drive out the air and allow the hydrofluoric acid, used in the next step of the process, to penetrate to all parts of the wood. Repeated boilings and additions of cold water hasten the process of driving out the air.
- 7 Plowman, A. B., The celloidin method with hard tissues. Bot. Gazette 37:456-461. 1904.